

**Anatoxin-a, Cylindrospermopsin,  
Microcystins/Nodularins, & Saxitoxins Report**  
*Project: Washoe County Community Services Department*

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Sample Receipt Date: 21 September 18  
Sample Condition: 7.6 °C upon arrival  
Report# 180920\_Washoe County Community Services Department  
Date Prepared: 24 September 18  
Prepared by: Kamil Cieslik

<u>Sample ID</u>	<u>Site/Description</u>	<u>Collection Date</u>	<u>Collection Time</u>
1	Lemmon Lake Inlet	20 September 18	1200

**Analytes:** Anatoxin-a (ANTX-A), Cylindrospermopsin (CYN), Microcystins/Nodularins (MCs/NODs), Saxitoxin (STX/PSTs)

### Sample Preparation

#### ***Water Sample Ultrasonication***

The sample was received and inverted for 60 seconds to mix. A subset of the sample was sonicated to release toxins and prepared for analyses.

## Analytical Techniques

### *Enzyme-Linked Immunosorbent Assay (ELISA)*

#### *MCs/NODs*

A microcystins/nodularins Adda ELISA (Abraxis) was utilized for the quantitative and sensitive congener-independent detection of MCs/NODs (US EPA Method 546 & Ohio EPA DES 701.0). The current method reporting limit is 0.15 ng/mL (ppb) based on kit sensitivity, dilution factors and initial demonstration of capability.

#### *STX*

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

### *Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)*

#### *ANTX-A*

The  $[M+H]^+$  ion for ANTX-A ( $m/z$  166) was fragmented and the product ions ( $m/z$  91, 131 & 149) were monitored. The sample response was compared to the LFSM response utilizing the quantification ion,  $m/z$  91. An external standard curve was used to determine LFSM returns.

#### *CYN*

The  $[M+H]^+$  ion for CYN ( $m/z$  416) was fragmented and the product ions ( $m/z$  194, 274, 336) were monitored. The sample response was compared to the LFSM response utilizing the quantification ion,  $m/z$  336. An external standard curve was used to determine LFSM returns.

## Quality Control

Table 1: LFSM QC samples prepared for analyses.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR	1.0	1 (Lemmon Lake Inlet)	LFSM	84%
CYN	0.1	1 (Lemmon Lake Inlet)	LFSM	82%
ANTX-A	0.1	1 (Lemmon Lake Inlet)	LFSM	107%
STX	0.2	1 (Lemmon Lake Inlet)	LFSM	80%

Additional Quality Control/Quality Assurance checks included method blanks, LFBs, and standard curves.

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

### Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit

**Summary of Results**

<b>Sample ID</b>	<b>MCs/NODs (ng/mL)</b>	<b>CYN (ng/mL)</b>	<b>ANTX-A (ng/mL)</b>	<b>STX (ng/mL)</b>
1 (Lemmon Lake Inlet)	ND	ND	ND	ND
<i>MRL (ng/mL)</i>	<i>0.15</i>	<i>0.05</i>	<i>0.05</i>	<i>0.05</i>
<i>Analyst Initials</i>	<i>KC</i>	<i>MA</i>	<i>MA</i>	<i>KC</i>
<i>Date Analyzed</i>	<i>9/24/18</i>	<i>9/21/18</i>	<i>9/21/18</i>	<i>9/21/18</i>

Submitted by:



Mark T. Aubel, Ph.D.

Date:

September 24, 2018

*The results in this report relate only to the samples listed above.  
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**Cyano**  
**LAB**